5/31/2016

Tuesday, May 31, 2016
2:43 PM

Making LB Plates

10g Bacto Tryptone
5g Yeast Extract
10g NaCl
7.5g Agar in each 500ml
In 1000 ml di water
Divided into two (500 ml) one with the following antibiotic

Added after autoclaving

Chloramphenicol - antibiotic blocks ribosome?
20 mg/ml (stock) --> final [c] 20 microg/mL
You just add 500 ml (the one without the antibiotic) to yield the

We're trying to create a tool, a promotor that is constructed into a vector,

Growth phase

We're trying to utilize different promotors and creating a vector that can turn them on and off

Goal:
Biobrick

Ecocyc

- Type in gene name and it'll tell you what the gene does

6/1/2016

Wednesday, June 1, 2016
1:10 PM

Roughly 6-9 promotors

T7 polymerase

gfp - illumination

We're picking promotors that change based on phase of growth

One or two promotors from each category

Put them in a plasmid in front of gfp
Put into e.coli
Some will immediately turn green (Early phase)
    Then they will turn off
Characterize each promotor based on gfp profile, when does the

Creating a plasmid that has all of these phases

Please review promotors and how they work
Please ask someone how promotors are identified and characterized?
If you're making a compound….
Put promotors of e.coli between genes of other organisms, you can get gene expression

Application of promotors

Webb outreach

Homework:
To-Do List

Summary of today:
We transformed bacteria, introducing already pre-made plasmids from the iGEM kit.

Agar plate formation: from 20,000 microg/mL to a batch of 500ml of 33 microgram

Extracted 10 microliters of E. coli DNA and placed in lb broth? I forget how much

Cold bath for 30

Incubated for 45 minutes

Then you place the samples in the warm water bath and heat shocked for 60 seconds

Use balls to streak the plates

6/2/2016

Thursday, June 2, 2016
2:02 PM

Developing promotors:

- Download genbank files for the promotor sequence
- Look for the gaps before the gene in question
- Take about 20 bp into the gene before and into the gene after
- Place Genbank download into A plasmid editor program (drag it directly into the program)

Place chlorphenicol in lb tubes and placed appropriate e. coli cells in tubes

6/3/2016

Friday, June 3, 2016
1:12 PM

Expression data

- MG1655

Genomic data

- MG1655

Strains in house

- Top10
- Dh5alpha
- (cloning strains) main goal is to build DNA plasmids, sometimes they have DNA restriction enzymes that help with cloning

You should use promotor from those strains

Cloning strains: DH5alpha

Expression strains: BL21

Wild Type Strains: unmodified

Build you get 1/100 efficiency

Ligation with cloning strains increases efficiency

Wild type (WT) --> cloning strain --> purify 100% still

Procedure for plasmid purification:

You have your cells and you want to pull out the plasmids

1. Spin/pellet cells --> centrifuge
2. Lyse and extract
    a. Usually three buffers that you use
    b. 250 microl Buffer 1 (resuspends) pipette (don't be too vigorous)
    c. 250 microl Buffer 2 (Lyses) invert tube (don't be too vigorous)
    d. 350 microl Buffer 3 (neutralizes) invert tube (don't be too vigorous)
3. Spin/purify for 10 minutes
    a. DNA resides in the liquid
4. Load supernatant into column
    a. Bind DNA
    b. Buffer A (inactivates enzymes that would eat up DNA)
    c. Wash buffer
    d. Elute with water
5. Results in pure plasmids

Nano Concentration measurement machine:

Make sure to blank with water

6/6/2016

Monday, June 6, 2016
10:13 AM

Read three articles assigned by Caroline

Write all procedures

iGEM Student Weekly Meeting

Goal for this week: isolating promotors

Promotors chosen:
3231
1304
1305
220
1224

Why would 1304 turn on off and then on again?

Autodesk Maya

Due Dates and Deadlines:

Data Analysis	September 5th
Outreach	July 1st know project, Oct 10th
Website	Design Aug 22, Oct 16th
Software	July 1, Oct 10
Poster	Oct 10
Presentation	Oct 10
Collaboration	June 20th

6/7/2016

Wednesday, June 8, 2016
2:19 AM

Cut DNA with restriction enzymes, prepared gel electrophoresis agar but didn't have time to run it

Homework:

Gene: starts around 146 (promotor)

5' --> 3'
atcctggcatgttgctgttg
62 - 81

Reverse Complement Primer

Primer (5'-->3')		length	%GC	Tm(°C)	self/other(max adj 3')
50 AGGATTGTCCTGCTGAACTG	69	20	50	56	8 4 1 /-----

6/8/2016

Wednesday, June 8, 2016
11:50 AM
Weekly Meeting 2

Test reporting genes?

6/10/2016

Friday, June 10, 2016
1:43 PM

Cleaned glassware

6/14/2016

Tuesday, June 14, 2016
1:46 PM

Chromosomal Extraction using the procedure followed on the following link:
https://mobio.com/media/wysiwyg/pdfs/protocols/12224.pdf

6/15/2016

Wednesday, June 15, 2016
12:36 PM

Group Meeting
Talked about plasmids
Group meeting Monday, come with articles

6/16/2016

Wednesday, June 15, 2016
1:02 PM

Prepared another excision set with 14A, 14C, and 1P
Use the following for this set

250 ng DNA
2 µL Cutsmart
0.5 µL EcoRI
0.5 SpeI
Water up to 20 µL

Everything below has been multiplied by 4.

You may find the procedure online. This is DNA digestion using restriction enzymes.

14A	14C	1P
7.12 µL DNA 8 µL CS 2 µL EcoRI 2 µL SpeI 60.88 µL diH_2O	8.76 µL DNA 8 µL CS 2 µL EcoRI 2 µL SpeI 59.24 µL diH_2O	7.00 µL DNA 8 µL CS 2 µL EcoRI 2 µL XbaI 61 µL diH_2O

You want the total volume to be 80 µl

After preparing, incubate for one hour in 37 degree using floating boat.

Then heat kill for 20 min in 80 degrees?

Then add phosphatase (4µL to 1P) then incubate at 37 for 30 min then heat kill

After finishing, save DNA in -20 for gel extraction another day.

Gel extraction happens after running the DNA through gel using gel electrophoresis

6/20/2016

Tuesday, June 21, 2016
12:06 AM

Ran 14A 14C and 1P through gels

Grad student helped us cut gel

Purifying tomorrow

6/22/2016

Wednesday, June 22, 2016
1:07 PM

Weekly Meeting
Discussed Applications
Lycopene

6/27/2016

Monday, June 27, 2016
2:52 PM

Weekly Student Meeting

7/11/2016

Monday, July 11, 2016
1:31 PM

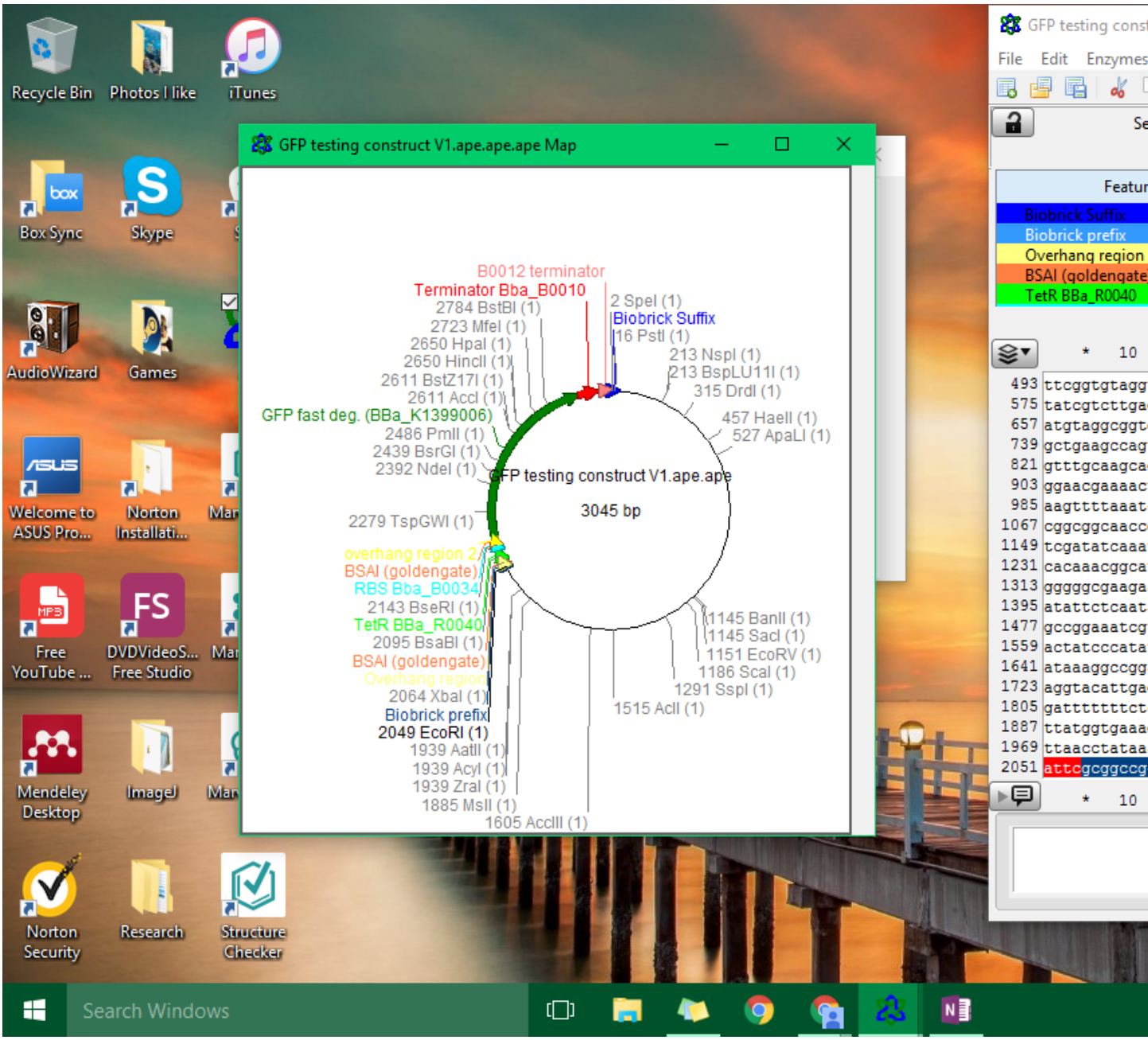
Redid 1304 and 2095 primer products

7/14/2016

Thursday, July 14, 2016
1:23 PM

Should be doing PCR Purification today

Possibly clone GFP PCR products into the linearized backbone however I'm uncertain as to what enzymes we should be using as we only have one thing to put in not two



By looking at this, I think we need to use EcoR1 and Spe1 or Pst1

The procedure online says to cut the linearized backbone with EcoR1 and Pst1 prior to ligation.

If everything goes well, please follow the procedure here to make the plasmids using the linearized backbone:
http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones

Then follow the transformation protocol.

7/15/2016

Thursday, July 21, 2016
3:04 PM

Worked on maya

7/18/2016

Monday, July 18, 2016
4:25 PM

Student Weekly Meeting:
Discussed outreach activities and target audience

7/20/2016

Wednesday, July 20, 2016
1:09 PM

Weekly Advisor Meeting:
What is provided for?
Photo consent forms
Teaching the principles of genetic engineering
Intent of Quad Day: recruiting
Testing GFP construct for this week

Email constructs from Interlab study to Courtney and advisors

Start ordering primers for lycopene pathway

Design more primers for the other promoters

Determine time frames to check using the periodic GFP testing method

PCR Colony test:
100 microliter of LB
    1 microliter as template for PCR
10 microliter PCR reaction

Order primers for PCR Colony tests

For Today:
Use XbaI and Pst1

All HF you can use Cutsmart

Better to run digestions overnight (2 hours is pretty good though)

7/21/2016

Thursday, July 21, 2016
3:15 PM

Executed transformation procedure again.

7/25/2016

Monday, July 25, 2016
1:29 PM

Transformation last Friday didn't work

Test whether or not cells are competent

The next step should be to see whether or not the DNA ligase procedure worked

Michelle gave us puc19 to see whether or not cells are competent
Ran transformation procedure again

Update: didn't seem to work

8/1/2016

Monday, August 1, 2016
1:18 PM

Weekly Student Meeting:

- Discussed sweet corn festival
- I will be responsible for finding a strawberry DNA extraction kit protocol and materials list

Things to do today:
Test new comp cells
Verify GFP part
    Use nanodrop
    IF concentration or part too small, redo PCR
Digest lycopene parts

Ran out of GFP…..
PCRed more GFP from the gBlock

August 31, 2016

Wednesday, August 31, 2016
10:22 AM

Weekly Advisor Meeting
Update weekly lab schedule for availabilities

September 13, 2016

Tuesday, September 13, 2016
2:56 PM

Today: I will be performing PCR to create the iGEM linearized Plasmid Backbone

Goal for today: Create 2 tubes of PCR product of 50 µL reactions

Protocol:

1. Dilute and resuspend the primers as needed since they just came in
2. The protocol, taken from the iGEM website is as follows:

PCR mix

I will actually be using the PCR Q5 Fidelity Master Mix

- 100 ul PCR Supermix High Fidelity
- 0.7 ul of SB-prep-3P-1
- 0.7 ul of SB-prep-2Ea
- 0.5 ul template DNA at 10 ng/ul
- Notes:

Do not use a sample of linearized plasmid backbones (PCRed) as a template,
The Registry uses BBa_J04450 as a template

Dilute the primers first
Template DNA - 262.4ng/µL
Diluted 1:100 to get 2.624 ng/µL
Prepared two tubes of 50µL reactions but may have added 2 µL instead of 1µL to Tube 1

PCR program

1. 94C/2min
2. 94C/30s
3. 55C/30s
4. 68C/3min
5. Repeat cycle (steps 2 to 4, 35 more times)
6. 68C/10min
7. Digest with DpnI enzyme: 2ul in 100ul reaction, incubate 37C/hour; heat kill 80C/20min

Row 1 is Tube 1
Row 2 is Tube 2
The machine says that the process may take up to 3 hours. I may not have time to complete the PCR cleanup procedure.

PCR cleanup

QIAquick PCR Purification

- Add 500 ul Qiagen buffer PB
- Spin through a column twice, discard flowthrough
- Wash 1x with 700 ul buffer PB
- Wash 2x with 760 ul buffer PE
- Discard liquid, spin dry at 17000g for 3 min
- Elute into a new tube twice with 50 ul of TE (100 ul total)

From <http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones>

Did not perform PCR Cleanup

September 28,2016

Wednesday, September 28, 2016
10:20 AM

Bi-Weekly Meeting

Troubleshooting -

Vector to plasmid ratio
1:3?

Count on having 100 ng of plasmid DNA per ligation reaction

Test to see if restriction enzymes work

Ligase works

- Pick part from MISC. DNA box
- Digest 1ug 200ng/uL --> 5 uL
    - PST1 -HF EcoR1 - HF Cutsmart
- Verify on gel
    - Ladder
    - Uncut sample

- PCR of backbone
    - Linearized backbone protocol
    - Primers have same name as in protocol, in our primer box

10/11/2016

Tuesday, October 11, 2016
1:04 PM

Experiment: Today, I'll be transforming competent dh5alpha *E. coli* cell with the Oxford part.

Purpose: The Oxford part contains the copper chelating genes. Presumably, we are replicating the Oxford part using bacteria (i.e. transformation)

Material Needed:

Plasmid: Tube of Oxford Copper Chelating Agent Genes (Csp1sf)
Cells: Competent dh5alpha (found in -80 degree freezer)

Ice for ice bath

Water bath for heat shock

Transformation Protocol:

1. Remember you need to thaw competent cells.
2. Dh5alpha cells for about 20 minutes on ice
3. Warm agar plates in 37C incubator.
4. Place 50µL of competent cells in 1.5 mL microcentrifuge tube.
5. Place 5µL plasmids in 1.5 mL microcentrifuge tube
6. Ice bath for 30 minutes
7. Heat shock (45 seconds) in 42 degree water bath
8. In the micro centrifuge, pipet 500µL of LB broth
9. Place tubes in flask
10. Secure to spinning incubator
11. Incubate for 45 minutes (shaking one, 37 degree)

12. Take 200µl (small amount)
13. Streak chlorophenicol plates using balls
14. Incubate overnight in the top part of the spinning incubator (37C)

Also used Nanodrop to measure the concentration and purity of the promoters 3321, 220, and 42 that underwent the golden gate process for insertion into the GPF construct. The data is as follows:

dsDNA_1		260	280	Conc ng/µl	Ratio	Sample ID
	A1	0.187	0.0974	187	1.92	220-1
	A2	0.1202	0.0622	120.2	1.93	3321-1
	B1	0.1826	0.0947	182.6	1.93	220-2
	B2	0.122	0.0649	122	1.88	3321-2
	C1	0.1685	0.0889	168.5	1.9	220-3
	C2	0.1601	0.0829	160.1	1.93	3321-3
	D1	0.1502	0.0765	150.2	1.96	220-4
	D2	0.0867	0.0462	86.7	1.88	3321-4
	E1	0.1468	0.0773	146.8	1.9	220-5
	E2	0.1444	0.0735	144.4	1.96	3321-5
	F1	0.1293	0.0661	129.3	1.96	220-6
	F2	0.1153	0.0611	115.3	1.89	3321-6
	G1	0.1818	0.0953	181.8	1.91	42-1
	G2	0.1832	0.093	183.2	1.97	42-2
	H1	0.2387	0.1205	238.7	1.98	neg
	H2	-0.0017	-0.0008	0	2.12	-

Plate with transformed bacteria are located in the top portion of the 37C spinning incubator. Need to be taken out

Purpose of the negative (H1): When you perform Golden Gate, the DNA of interest gets inserted into the backbone. We have the negative control because we're just seeing if the DNA actually got incorporated. If the concentrations are the same, then the new DNA was never incorporated.

We will also be preforming restriction digest to verify that our promoters actually got inserted into the plasmid.

10/12/2016

Wednesday, October 12, 2016
10:08 AM

Bi-weekly meeting:
Poster
Presentation - get started
T-shirt

10/18/2016

Tuesday, October 18, 2016
1:41 PM

Experiment: Today, I will be running PCR tubes of backbone through gel electrophoresis. Then, I will be going through the linearized backbone protocol to

Purpose:

I will begin with the verification of the PCR products using the gel electrophoresis protocol. The band should be 2 kb.

PCR Specific Procedure (tweak if necessary for other procedures)

1. For PCR we'll use a 1% gel
2. Add **.5g** of agarose to **50 µL** of TAE buffer (use one of the bottles with a yellow cap)
3. Microwave bottle with yellow cap for **1 minute**. Make sure to **occasionally stir** the bottle to make the buffer solution more consistent
4. Allow the bottle to cool for **5 minutes**.
5. Add **6 µL** of advanced midori green dye (found in the 4 degree room).
6. Pour liquid into gel box (make sure the plate is not tilted and that the liquid level doesn't go over the height level of the wells)
7. Allow roughly **30 minutes** to harden the gel
8. Use the purple (or whatever concentration/color loading dye) loading dye, which happens to be **6X** so add about **1µL** of dye to **5µL** of DNA taken from the already PCRed tubes in however many **new** tubes
9. Add a little bit of loading dye (**5µL** should be sufficient) into the first well and the well adjacent to the 4th well
10. As for right now, we're only doing this to see if our primers are working, so the entire amount of DNA from all three tubes can be placed in the 2nd, 3rd, and 4th well.
11. Run at **120V** for **20 minutes**
12. Image

While we are waiting, we will begin purifying the PCRed linearized backbone.

Protocol is as follows:

PCR cleanup

QIAquick PCR Purification
Add 500 ul Qiagen buffer PB
Spin through a column twice, discard flowthrough
Wash 1x with 700 ul buffer PB
Wash 2x with 760 ul buffer PE
Discard liquid, spin dry at 17000g for 3 min
Elute into a new tube twice with 50 ul of TE (100 ul total)

From <http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones>